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Preliminary

Free-radical Polymerization of Acrylamide by Manganese Peroxidase Produced by the White-rot Basidiomycete *Bjerkandera adusta*[*1]

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Introduction

Manganese peroxidase (MnP), one of the lignin-degrading enzymes produced by the white-rot basidiomycetes, catalyzes oxidation of Mn(II) to Mn(III) in the presence of hydrogen peroxide. Mn(III) withdraws one electron from phenolic compounds including lignin polymer and related oligomeric and monomeric phenolics to generate phenoxy radicals[1]. Alternatively, radicals undergo condensation to give the polymerization products[2]. Recently, we reported the synthesis of dehydrogenation polymers from coniferyl and sinapyl alcohols[3] and polyguaiacol from guaiacol[4] by MnP. This ability to generate radicals is considered to apply to the polymerization of vinyl compounds via a radical additive reaction. This paper deals with the polymerization of acrylamide using a lignin-degrading enzyme, MnP.

Materials and Methods

MnP was prepared from the culture of *Bjerkandera adusta* (K-2679)[5]. Horseradish peroxidase (HRP) was used also in the polymerization reaction. The molecular weight of polyacrylamide was measured by gel permeation chromatography (GPC) using a Shodex SB-806 prepacked column. ESR spectra were recorded on a JEOL model JES-FR30 spectrometer. ^{13}C- and ^{1}H-nuclear magnetic resonance (NMR) spectrometry was carried out using a JEOL model JNM-LA400MK. A Thermal Gravimetric Analyzer (TGA) and a Differential Scanning Calorimeter (DSC) (TA-Instrument, USA) were used.

Polymerization was started by adding 0.5 ml of a 40 mM hydrogen peroxide solution to a solution containing MnP (500 units), 0.2 mM $MnSO_4$, 20 mM lactate buffer (pH 4.5), 0.17 mM 2, 4-pentanedione and 700 mM acrylamide in 20 ml. The reaction was carried out at room temperature. After 12 hours, the reaction mixture was introduced into a large excess of methanol. The precipitate formed was collected by filtration, washed with methanol and dried under vacuum at 45°C. It was then weighed to estimate the polymer yield.

Results and Discussion

Enzymatic polymerization of acrylamide was examined using MnP in the presence of 2, 4-pentanedione as a radical initiator. In this reaction, the initiator was essential for the polymerization. The yield of the polymer was 96% against added acrylamide. The polymerization did not occur without addition of MnP, Mn(II) or hydrogen peroxide, indicating polymerization by MnP. Acrylmide was also polymerized in a reaction using HRP and initiator. A non-enzymatic reaction using Fe-ASP system containing 68 mM $FeCl_2$, 0.17 mM ammonium persulfate and 700 mM acrylamide gave the polymerization product as well. Yield, weight average molecular weight (Mw), number average molecular weight (Mn) and degree of dispersion of the polymer (d) obtained in the three conditions are summarized in Table 1.

In the ^{13}C-NMR spectrum, chemical shifts of methine (CH) (129.2 ppm) and methylene (CH_2) (130.2 ppm) in acrylamide moved to the region 42.9–43.8 ppm and 35.2–37.5 ppm in the polymer, respectively, whereas the carbonyl (C=O) carbon moved slightly to a low magnetic field. No other signal was detected in a spectrum, indicating that the polymer has a straight-chain structure in which acrylamide is a repeating unit. Chemical shifts of methine, methylene and carbonyl carbons of the polymers synthesized by HRP and Fe-ASP were the same as those of MnP-polymerized products.

Microtacticity analysis carried out by ^{13}C-NMR spectrometry indicated that the polymer was atactic (Fig. 1). Polymers synthesized by HRP showed similar microtacticity. The tacticity of MnP- and HRP-mediated polacrylamide was found to be consistent with the analytical data obtained from Fe-ASP-mediated poly-

Table 1. Yield and molecular weight of polyacrylamide synthesized by MnP, HRP and the Fe-APS reaction system.

Reaction system	Yield (%)	Mw	Mn	
MnP	96	155,000	40,500	3.8
HRP	97	67,500	20.000	3.4
Fe-APS	91	13,000	8,100	1.6

Mw=weight average olecular weight, Mn=number average molecular weight, d=Mw/Mn=degree of dispersion.

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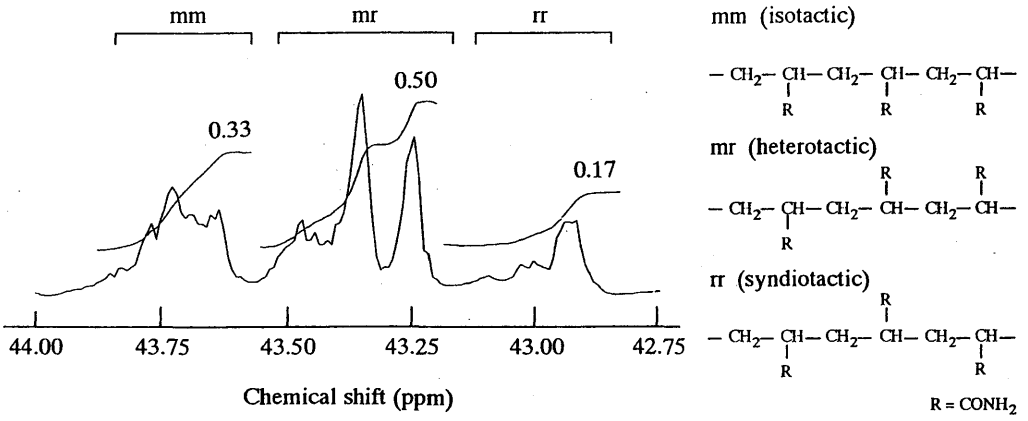

Fig. 1. ^{13}C-NMR spectra of acrylamide - Expansion of the methane carbon region of polyacrylamide and assignment of triad tacticity of methane carbons.

acrylamide[6].

The change of radical spin resonance in the reaction of acrylamide polymerization was measured by ESR spectrometry using POBN as a radical trapping reagent. The ESR spectrum without added acrylamide consisted of a triplet of doublets. From the features of the hyperfine splitting in the spectrum, the radical species was assigned a carbon-centered configuration. When the substrate acrylamide was added to this reaction system, additional signals appeared besides those that appeared in the absence of acrylamide. These signals were predominant after 30 minutes reaction, whereas signals of POBN adduct of 2, 4-pentanedione disappeared. Thus, ESR analysis indicated that the first step of the polymerization is the generation of a carbon-centered radical of 2, 4-pentadione, which promotes propagation of the polymer chain.

The thermal stability of the polyacrylamide was analyzed by TG from room temperature to 1,000°C in air. Weight loss until 110°C was considered to be caused by the removal of water from the polymer. The decomposition of polyacrylamide started at 242°C and was completed at 510°C. DSC showed the glass transition temperature was 190°C. However, a clear melting point was not observed. These properties suggested that polyacrylamide could be used as a thermoplastic resin.

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